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## Enhanced activation of rhesus T cells by vectors encoding a triad of costimulatory molecules (B7-1, ICAM-1, LFA-3).

Shankar P, Schlom J, Hodge JW.

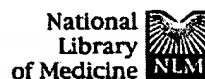
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Since the rhesus is often used as a "gatekeeper" model for the evaluation of malaria and simian immunodeficiency virus (SIV)/HIV vaccines, the identification of strategies to enhance the activation of rhesus T cells would potentially aid in the generation of more potent vaccines directed against these infectious agents. Several molecules normally found on the surface of professional human APCs are capable of providing the second signals critical for T cell activation: B7-1 (CD80), ICAM-1 (CD54), and LFA-3 (CD58). With the exception of B7, T cell costimulatory molecules in the rhesus have not been identified. We have recently designed and characterized both recombinant vaccinia and recombinant avipox vectors containing the transgenes for a triad of human T cell costimulatory molecules (B7-1, ICAM-1, LFA-3; designated TRICOM). Here, we demonstrate the enhanced activation of rhesus T cells stimulated with rhesus APCs infected with TRICOM vectors in the presence of signal 1. Infection with TRICOM vectors led to significant improvement of APC capabilities in terms of reduction of the amount of signal 1 needed to activate naive T cells, and reduction in the amount of APCs required to activate T cells using a constant amount of signal 1. Antibody blocking studies demonstrated that each of the three costimulatory molecule transgenes contributed to the enhanced proliferation of T cells. TRICOM-enhanced T cell activation was shown to correspond to increases in type 1 cytokines and a reduced level of apoptosis. TRICOM-infected autologous B cells from rhesus immunized with either an SIV vaccine or a malaria vaccine stimulated significantly greater levels of IFN-gamma in response to specific peptide than stimulation with uninfected autologous B cells or B cells infected with wild-type vector. The ability to augment immune responses using poxvirus-based vaccines containing multiple costimulatory molecule transgenes can now be addressed in the rhesus macaque model.

PMID: 11738738 [PubMed - indexed for MEDLINE]



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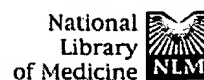
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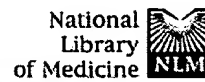
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Cancer Detect Prev. 2002;26(4):275-91. Review.  
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Vector-based vaccine/cytokine combination therapy to enhance induction of immune responses to a self-antigen and antitumor activity.  
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- ☐ **4:** [Rad AN, Schlom J, Hodge JW.](#) [Related Articles, Links](#)

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Crit Rev Oncol Hematol. 2001 Jul-Aug;39(1-2):43-57.  
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- ☐ **5:** [Grosenbach DW, Barrientos JC, Schlom J, Hodge JW.](#) [Related Articles, Links](#)

Synergy of vaccine strategies to amplify antigen-specific immune responses and antitumor effects.  
Cancer Res. 2001 Jun 1;61(11):4497-505.  
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- ☐ **6:** [Hodge JW, Grosenbach DW, Rad AN, Giuliano M, Sabzevari H, Schlom J.](#) [Related Articles, Links](#)

Enhancing the potency of peptide-pulsed antigen presenting cells by vector-driven hyperexpression of a triad of costimulatory molecules.  
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PMID: 11348723 [PubMed - indexed for MEDLINE]

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Int J Cancer. 2000 Feb 15;85(4):508-17.  
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Cancer Res. 1999 Nov 15;59(22):5800-7.  
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- ☐ **9:** [Schlom J, Hodge JW.](#) [Related Articles, Links](#)  
The diversity of T-cell co-stimulation in the induction of antitumor immunity.  
Immunol Rev. 1999 Aug;170:73-84. Review.  
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Comparative studies of a retrovirus versus a poxvirus vector in whole tumor-cell vaccines.  
Cancer Res. 1999 Oct 15;59(20):5106-11.  
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- ☐ **11:** [Lorenz MG, Kantor JA, Schlom J, Hodge JW.](#) [Related Articles, Links](#)  
Induction of anti-tumor immunity elicited by tumor cells expressing a murine LFA-3 analog via a recombinant vaccinia virus.  
Hum Gene Ther. 1999 Mar 1;10(4):623-31.  
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The use of combination vaccinia vaccines and dual-gene vaccinia vaccines to enhance antigen-specific T-cell immunity via T-cell costimulation.  
Vaccine. 1999 Feb 26;17(7-8):893-903.  
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Construction and characterization of a recombinant vaccinia virus expressing murine intercellular adhesion molecule-1: induction and potentiation of antitumor responses.  
Hum Gene Ther. 1997 May 1;8(7):851-60.  
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Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses.  
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Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules.

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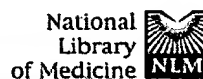
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## Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules.

Hodge JW, Abrams S, Schlom J, Kantor JA.

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Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

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Activation of T cells requires at least two signals: an antigen-specific signal delivered through the T-cell receptor and a costimulatory signal mediated through molecules designated B7-1 and B7-2. Previous studies have shown that introduction of B7-1 and B7-2 into tumors using retroviral vectors has led to enhanced antitumor effects. A limiting factor for potential clinical applications using this approach is the low efficiency of infection of retroviral vectors and consequent manipulations of infected cells. Vaccinia virus thus represents an alternative vector for B7 gene expression in tumor cells. In this report we describe the construction and characterization of recombinant vaccinia viruses containing the murine B7-1 and B7-2 genes (designated rV-B7-1 and rV-B7-2). Infection of BSC-1 cells with these constructs results in rapid and efficient cell surface expression of both B7-1 and B7-2 (> 97% of cells at 4 h). Infection of murine carcinoma cells with low multiplicity of infection of wild-type vaccinia virus leads to the death of the host following tumor transplantation. In contrast, inoculation of rV-B7-1- or rV-B7-2-infected tumor cells into immunocompetent animals resulted in no tumor growth. These studies demonstrate the utility of recombinant vaccinia viruses to deliver B7 molecules to tumor cells for potential gene therapy and recombinant approaches to cancer immunotherapy.

PMID: 7522961 [PubMed - indexed for MEDLINE]

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## Nonreplicating recombinant vaccinia virus encoding human B-7 molecules elicits effective costimulation of naive and memory CD4+ T lymphocytes in vitro.

Marti WR, Zajac P, Spagnoli G, Heberer M, Oertli D.

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Department of Surgery, University Hospital of Basel, Switzerland.

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We constructed recombinant vaccinia viruses (recVV) encoding the human T-cell costimulatory molecules B7-1 and B7-2. To abrogate the vaccinia virus transcription termination signal for early genes, the cDNA of B7-1 had to be modified by a T through C sense mutation at position 766. Upon infection with replication incompetent and noncytopathic recVV, several tumor cell lines as well as cultured human fibroblasts expressed the costimulatory molecules. All these cells were capable of providing effective costimulation for proliferation of resting CD4(+) T-cells after infection with recVV encoding B7 molecules. The costimulatory effect could be blocked with CTLA-4 IgG fusion protein, the soluble ligand for B7. RecVV-induced overexpression of B7 on syngeneic EBV-transformed lymphoblastoid B-cells was able to costimulate the proliferative response of CD4(+) memory cells against VV antigens. The possibility of easily engineering a variety of human cells using recVV encoding human B7 molecules holds implications for the future design of vaccination strategies.

PMID: 9268498 [PubMed - indexed for MEDLINE]

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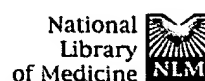
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## Enhanced generation of cytotoxic T lymphocytes using recombinant vaccinia virus expressing human tumor-associated antigens and B7 costimulatory molecules.

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**Zajac P, Schutz A, Oertli D, Noppen C, Schaefer C, Heberer M, Spagnoli GC, Marti WR.**

Department of Surgery, University of Basel, Center for Teaching and Research, Switzerland.

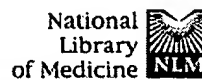
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In this work, we addressed the possibility to enhance the "in vitro" generation of CTLs recognizing tumor-associated antigens (TAAs) by using an inactivated recombinant vaccinia virus encoding B7.1 and B7.2 costimulatory molecules (rVV-B7.1/2). Antigen presenting cells (APCs) infected by rVV-B7.1/2 and pulsed with MART-1/Melan-A27-35 HLA-A2.1-restricted peptide induced significantly higher specific cytotoxic activity than peptide-loaded APCs infected by wild-type VV, both in VV-sensitized and naive donors. When APCs were infected with a rVV encoding both MART-1/Melan-A27-35 and B7-1/2 (rVV-B7.1/2-M), a significantly more effective CTL generation was observed as compared with cultures stimulated by APCs infected with a rVV encoding the TAA epitope only (rVV-M). These enhancing effects were detectable irrespective of a previous VV-specific sensitization. Most importantly, fibroblasts, devoid of antigen-presenting capacity upon peptide pulsing or infection with rVV-M, could be turned into effective APCs after infection by rVV encoding TAA epitopes and costimulatory molecules. In these experiments, by using separate recombinant viral constructs, we observed a predominant role of B7-1 as compared with B7-2 in the induction of TAA-specific CTLs. Taken together, our data indicate that replication-incompetent rVV encoding TAA epitopes and costimulatory molecules are able to induce highly effective generation of tumor-specific CTLs. Therefore, these vectors could represent valuable clinical tools for immunotherapy of melanoma patients.

PMID: 9788602 [PubMed - indexed for MEDLINE]

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## Non-replicating recombinant vaccinia virus encoding murine B-7 molecules elicits effective costimulation of naive CD4<sup>+</sup> splenocytes in vitro.

Oertli D, Marti WR, Norton JA, Tsung K.

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Department of Surgery, University Hospital of Basel, Switzerland.

Using a series of new insertion/expression vectors, we constructed a set of recombinant vaccinia viruses (recVV) encoding the murine T cell costimulatory molecules mB7-1 or mB7-2, or both together in the same construct. On infection with replication incompetent and non-cytopathic recVV, several tumour cell lines expressed the respective molecules and bound to CTLA-4. The highest binding capacity was found when both mB7 molecules were co-expressed. Mouse B16.F10 melanoma cells expressing mB7-1 or mB7-2 provided effective costimulation for proliferation of resting CD4<sup>+</sup> T cells in the presence of concanavalin A and plate-bound anti-T cell receptor antibodies, respectively. If mB7-1 and mB7-2 were delivered together on the same cell, the proliferative response of CD4<sup>+</sup> T cells increased further. The costimulatory effect could be blocked with CTLA-4, the soluble ligand for B7 molecules. The possibility of engineering tumour cells using recVV holds implications for the future design of vaccination strategies.

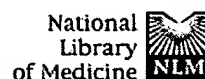
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## The role of B7-1 and LFA-3 in costimulation of CD8+ T cells.

Parra E, Wingren AG, Hedlund G, Kalland T, Dohlsten M.

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The Wallenberg Laboratory, Department of Cell and Molecular Biology, University of Lund, Sweden.

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This study compares the ability of LFA-3 (CD58) and B7-1 (CD80) ligands to provide costimulatory signals for superantigen (SAg)-stimulated CD8+ and CD4+ T cells. We show that B7-1 and LFA-3 costimulation activate CD8+ T cells to proliferation, cytokine production (IL-2, TNF, and IFN-gamma), and cytotoxicity. A long-lasting proliferative response was observed after combined DR/B7-1/LFA-3 costimulation. Detailed analysis of SEA-activated CD8+ T cells revealed that maximal production of IFN-gamma was seen in LFA-3-costimulated cells, while production of IL-2 was mainly induced after B7-1 costimulation. A fivefold increase in the IFN-gamma production was observed when activated CD8+ T cells were costimulated with Chinese hamster ovary (CHO)-DR/LFA-3 cells compared with the secretion induced by CHO-DR/B7-1. In contrast, SEA-treated CD4+ T cells costimulated with B7-1 or LFA-3 gave rise to a similar production of IFN-gamma, suggesting a preferential function for the CD2/LFA-3 pathway in the regulation of IFN-gamma in CD8+ T cells. Moreover, the generation of CTL was supported similarly by B7-1 and LFA-3 costimulation, but not by CHO-DR cells. We conclude that ligation of the CD28 and CD2 receptors mediate distinct effect on CD8+ and CD4+ T cell effector functions.

PMID: 8992978 [PubMed - indexed for MEDLINE]

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☐ 1: J Immunol 1994 Sep 15;153(6):2479-87

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## **Costimulation of human CD4+ T lymphocytes with B7 and lymphocyte function-associated antigen-3 results in distinct cell activation profiles.**

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**Parra E, Wingren AG, Hedlund G, Bjorklund M, Sjogren HO, Kalland T, Sansom D, Dohlsten M.**

Wallenberg Laboratory, Department of Tumor Immunology, University of Lund, Sweden.

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This study describes the distinct roles of B7 and LFA-3 in the regulation of T cell responses. Activation of CD4+ T cells with Chinese hamster ovary (CHO)-DR4/B7 and CHO-DR4/LFA-3 cells that present the superantigen staphylococcal enterotoxin A resulted in significant T cell proliferation and substantial production of TNF and IFN-gamma. Strong IL-2 production was recorded in B7-costimulated, but not LFA-3-costimulated, cultures. The presence of B7 induced a more vigorous and prolonged proliferative T cell response compared with LFA-3 costimulation. In contrast, LFA-3 was more efficient than B7 in mediating cell adhesion of CD4+ T cells. Costimulation with the CHO-DR4/B7/LFA-3 triple transfectant resulted in enhanced cell adhesion, proliferation, and cytokine production compared with either DR4/B7 or DR4/LFA-3 alone. Optimal production of IL-2 by naive and memory CD4+ T cells was seen only when cells were costimulated with B7, whereas IFN-gamma production was induced in memory cells by both LFA-3 and B7. The Jurkat T cell line responded to CHO-DR4/B7/LFA-3 in a manner similar to peripheral blood CD4+ T cells. Reverse transcriptase-PCR analysis of Jurkat cells stimulated with staphylococcal enterotoxin E and the different CHO transfectants revealed that the cooperative effect of B7 and LFA-3 on IL-2 production was also seen at the mRNA level. The large amounts of IL-2 produced by B7 costimulation indicate a paracrine function of the B7/CD28 pathway, whereas the LFA-3/CD2 pathway provides strong adhesion and may facilitate autocrine T cell expansion. Combined expression of the B7 and LFA-3 molecules seems to provide an optimal Ag-presenting function that ensures strong adhesion and optimal signal transduction.

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